Last lecture:

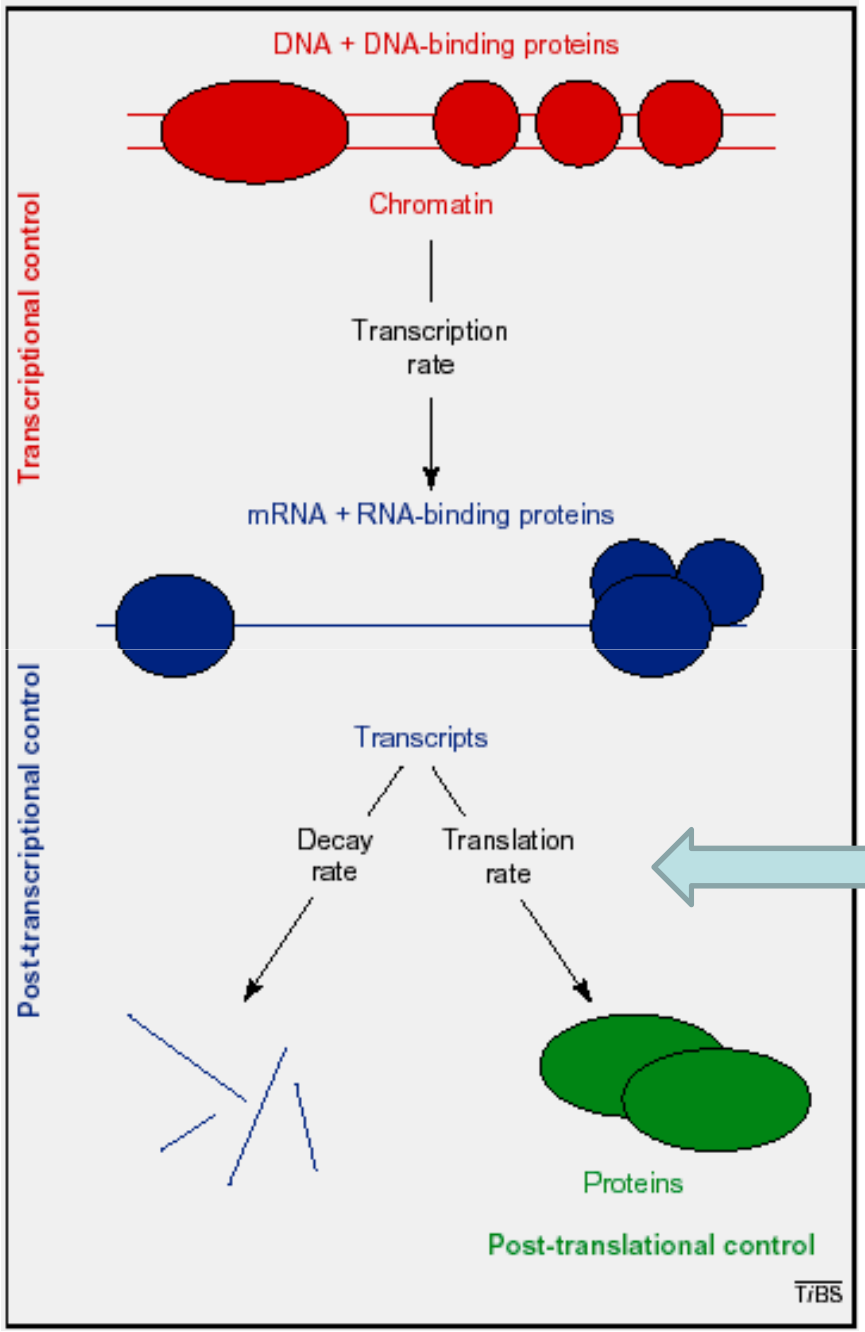
Mechanisms of nuclear export and mRNA localization in the cell

Complex structure of regulatory RNA elements and proteins

Today’s lecture :

will deal with a tightly connected question that is the regulation of mRNA stability and translation

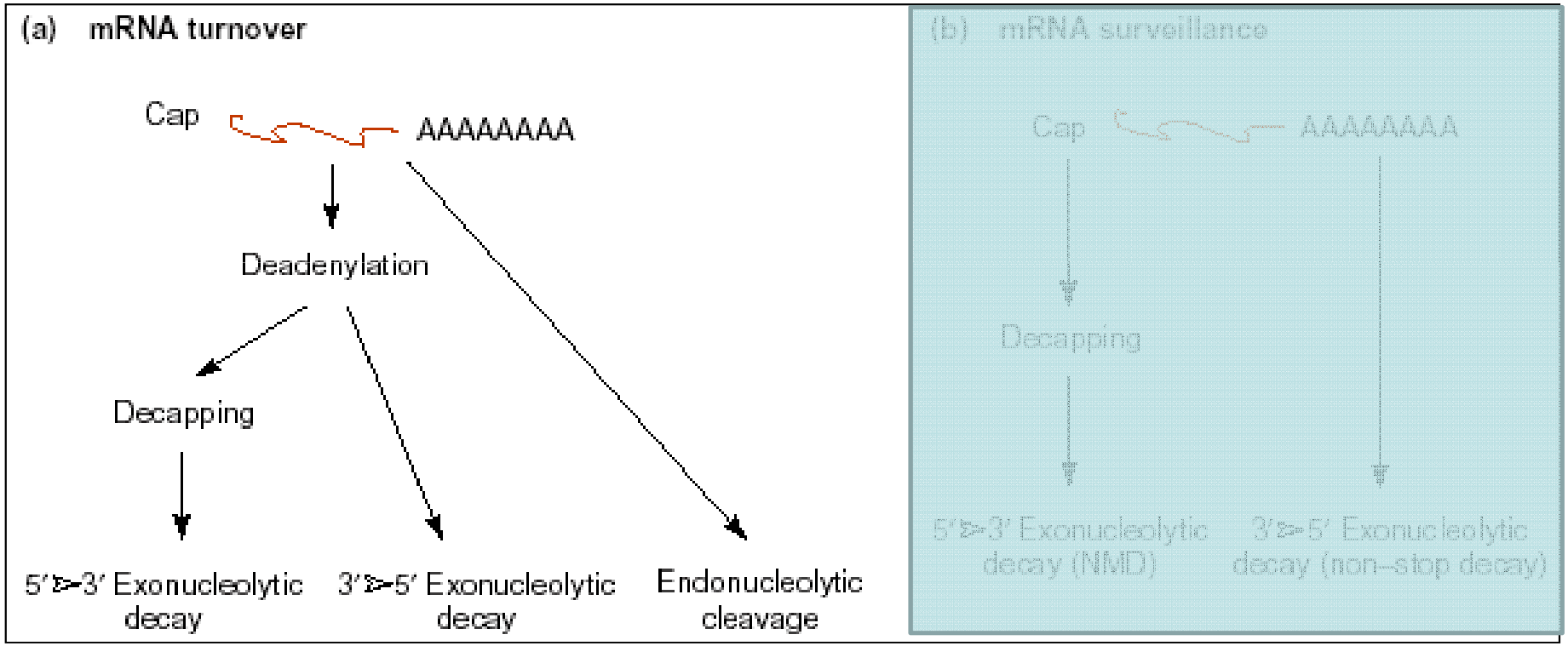
The main connection is that mRNA localization also depends on translation inhibition (regulation) and local degradation/stabilization of specific mRNAs.



POST-TRANSCRIPTIONAL REGULATION OF GENE EXPRESSION

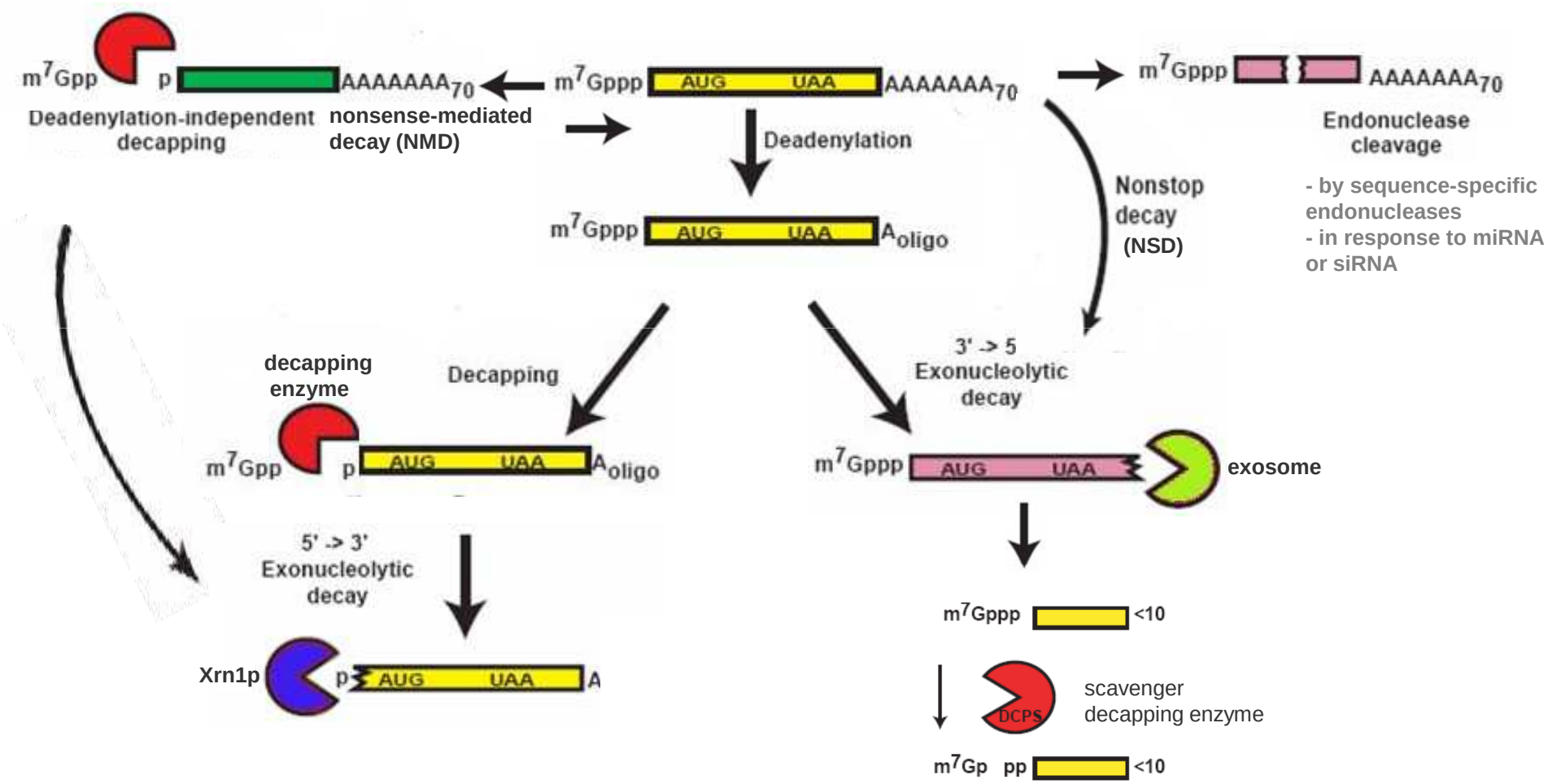
mRNA TURNOVER AND DECAY

From: Mata et al.(2005) Trends Biochem Sci 30:506-514.



From: Mata et al.(2005) Trends Biochem Sci 30:506-514.

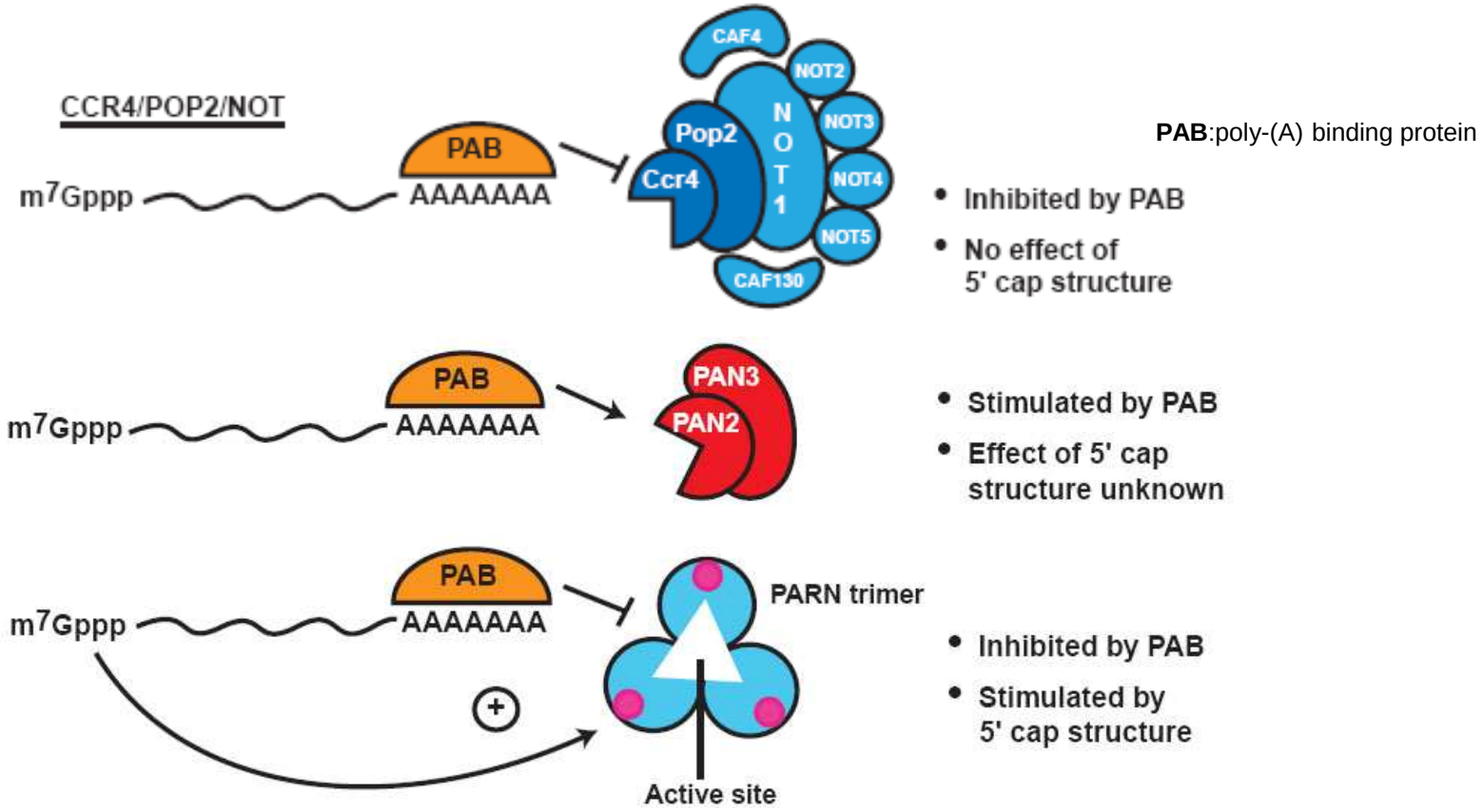
Pathways of eukaryotic mRNA turnover



Parker R. & Song H., 2004

control of deadenylation

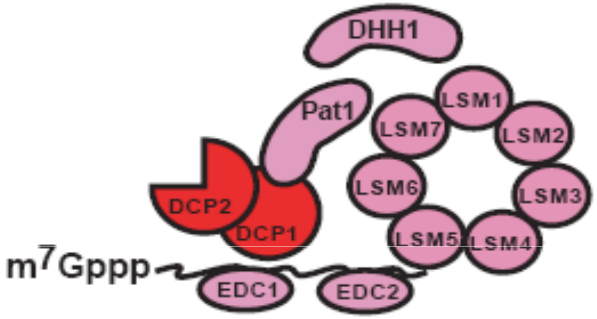
mRNA deadenylases have different biochemical properties and activation/inhibition molecules and structures and prefer different mRNA substrates



Parker R. & Song H., 2004

Differential recruitment of decapping enzymes to different mRNA substrates

Normal mRNA decapping

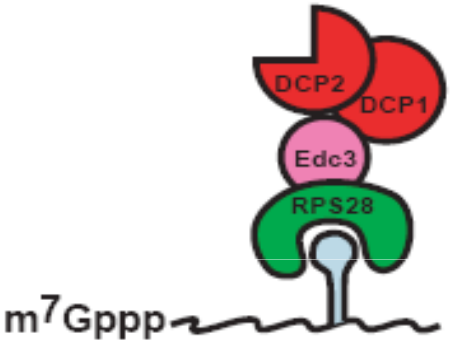


direct interaction DCPs-mRNA

Nonsense-mediated decay

DCPs recruitment to the mRNA via interactions with **Upf1P**

Regulation of RPS28A mRNA



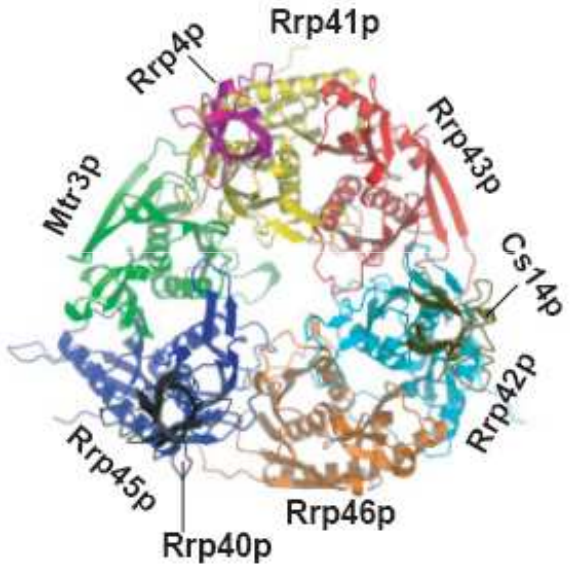
autoregulatory process: the Rps28 protein binds a stem loop in the 3'UTR of the mRNA and recruits DCPs through the Edc3p protein.

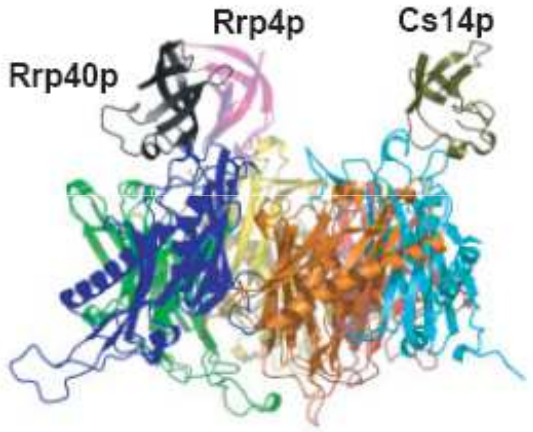
Parker R. & Song H., 2004

3’-5’ degradation of the mRNA body: the exosome

is a large protein complex containing multiple 3’-5’-exonucleases

the core is composed of 9 subunits that form ringlike structures and use phosphate as an attacking group during RNA digestion, producing NDPs





Parker R. & Song H., 2004

- six of the nine core exosome subunit contain RNase PH domain

- three subunits possess KH and S1 RNA-binding domains

- one additional exosome subunit is Rrp44, a 3’ hydroxylase of RNaseD family

Mechanisms of exosome recruitment to different mRNA substrates

General mRNA degradation

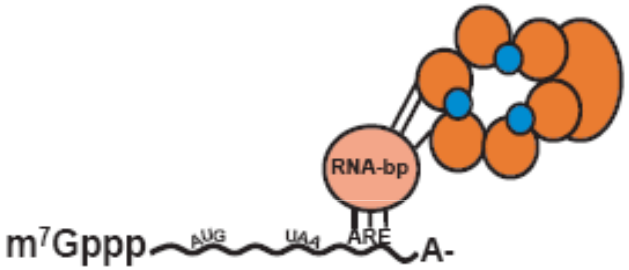
exosome interacts with the heteromeric complex of **Ski2p, Ski3p, Ski8p via Ski7p**

Ski2p is an ATP-dependent helicase that removes RNA structures or protein to allow exosome digestion and promotes the entry of the mRNA into the central cavitiy of the exosome

Parker R. & Song H., 2004

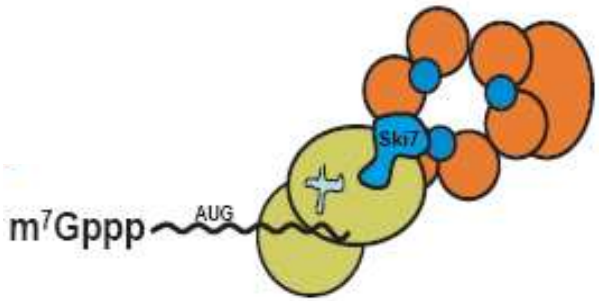
ARE-mediated decay

see next slide



specific ARE-binding proteins (TTP and KuR) physically interact with the exosome and recruit it to the mRNA

Nonstop decay



G-protein domain of Skip7 interacts with ribosomes stalled at the 3'end of the transcript and with the exosome, thus recruiting it to the mRNA

Regulating mRNA decay by *cis*-acting elements: the ARE elements

- are A/U-rich elements found in the 3'-UTR of some mRNAs encoding cytochines, proto-oncogenes and growth factors
- are defined by their ability to promote rapid deadenylation-dependent mRNA decay
- their sequence requirements are only loosely conserved

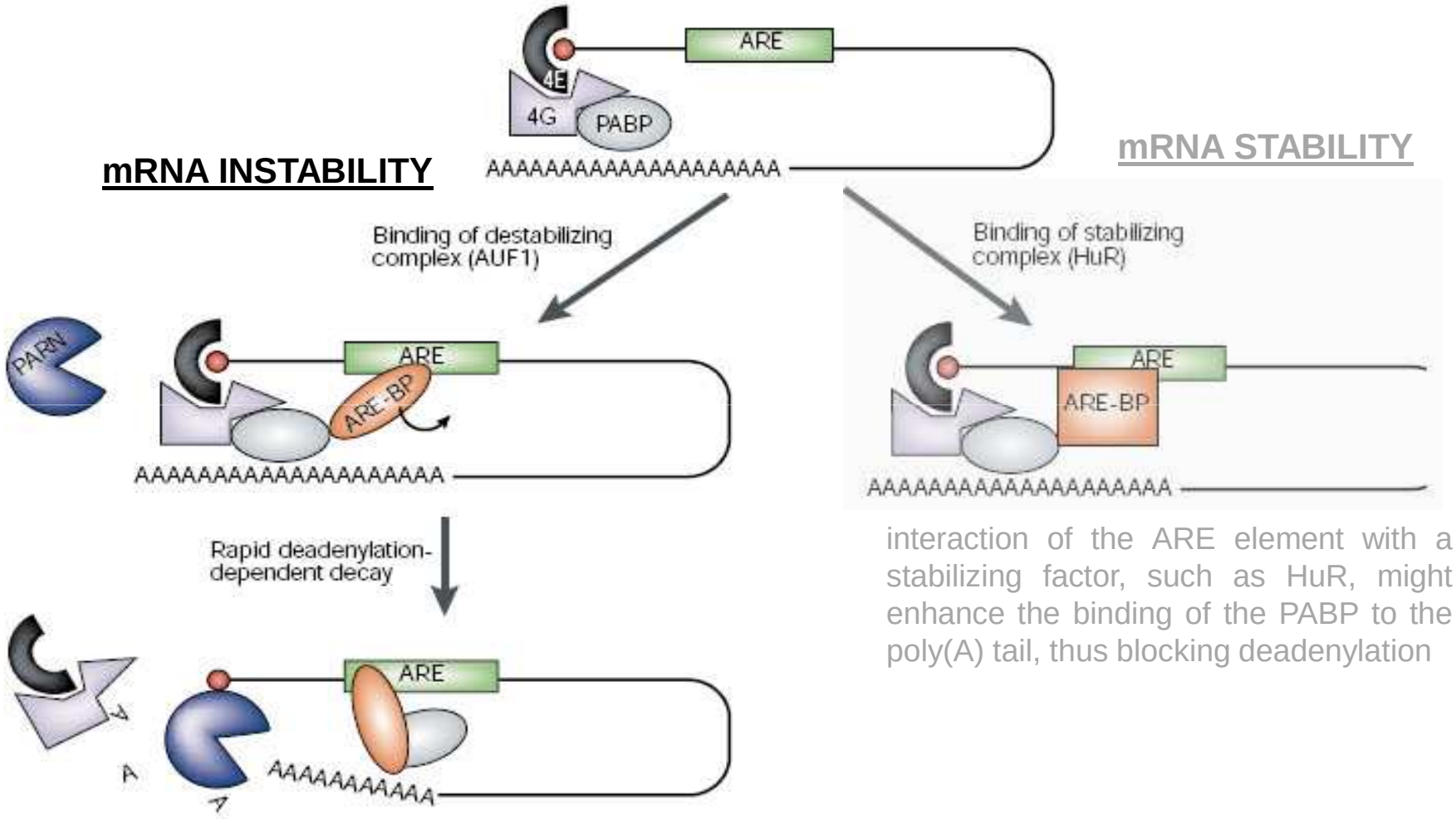
Group	Motif	Examples
I	WAUUUAW and a U-rich region	c-fos, c-myc
IIA	AUUUAUUUAUUUAUUUAUUUA	GM-CSF, TNF-α
IIB	AUUUAUUUAUUUAUUUA	Interferon-α
IIC	WAUUUAUUUAUUUAW	cox-2, IL-2, VEGF
IID	WWAUUUAUUUAWW	FGF2
IIE	WWWWAUUUAWWWW	u-PA receptor
III	U-rich, non-AUUUA	c-jun

Wilusz J.C. et al., 2001

ARE-binding proteins recognize these elements and, in conjunction with other proteins, will guide the mRNA to exosome degradation.

ARE-binding proteins

Many ARE-binding protein have been identified and have either *negative* or in some cases *positive* effect on processes such as stability, translation and subcellular localization of the mRNA



interaction of the ARE element with a destabilizing factor, such as AUF1, might promote rapid deadenylation by reducing the affinity of the poly(A) binding protein (PABP) for the poly(A) tail

interaction of the ARE element with a stabilizing factor, such as HuR, might enhance the binding of the PABP to the poly(A) tail, thus blocking deadenylation

Wilusz J.C. et al., 2001

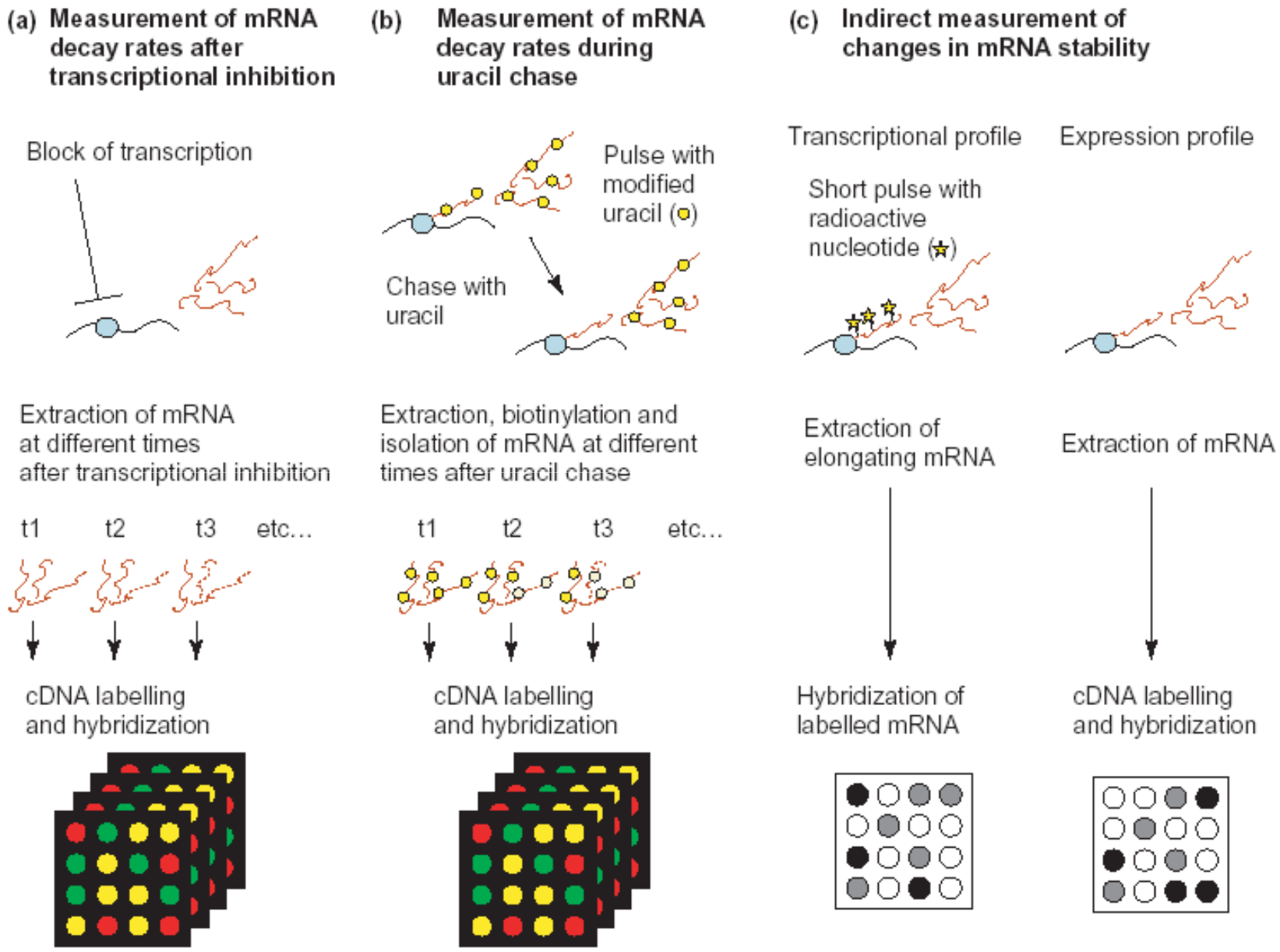
Again, we have:

1) quite common *cis*-elements that are recognized by common RBP

2) more specific cis-elements that can be regulated by more specific proteins

3) also tissue-specific

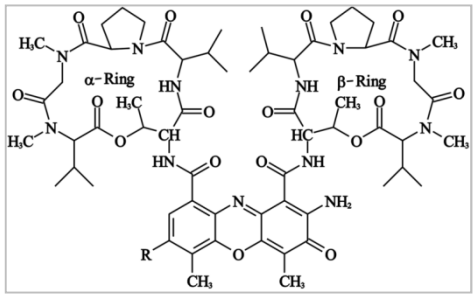
Are there methods to measure mRNA stability and degradation ?



From: Mata et al.(2005) Trends Biochem Sci 30:506-514.

How to measure mRNA half-life on single genes

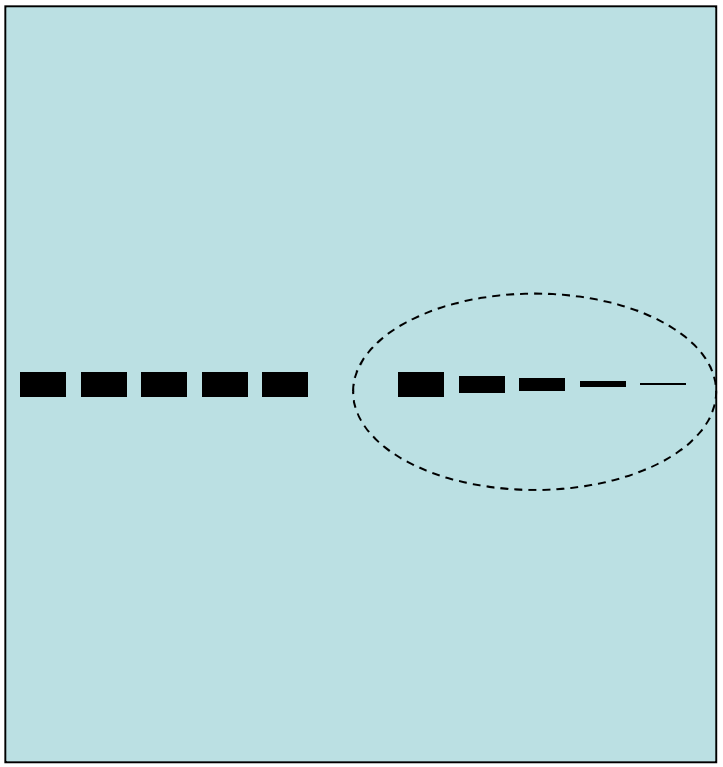
Cells treated with Actinomycin D



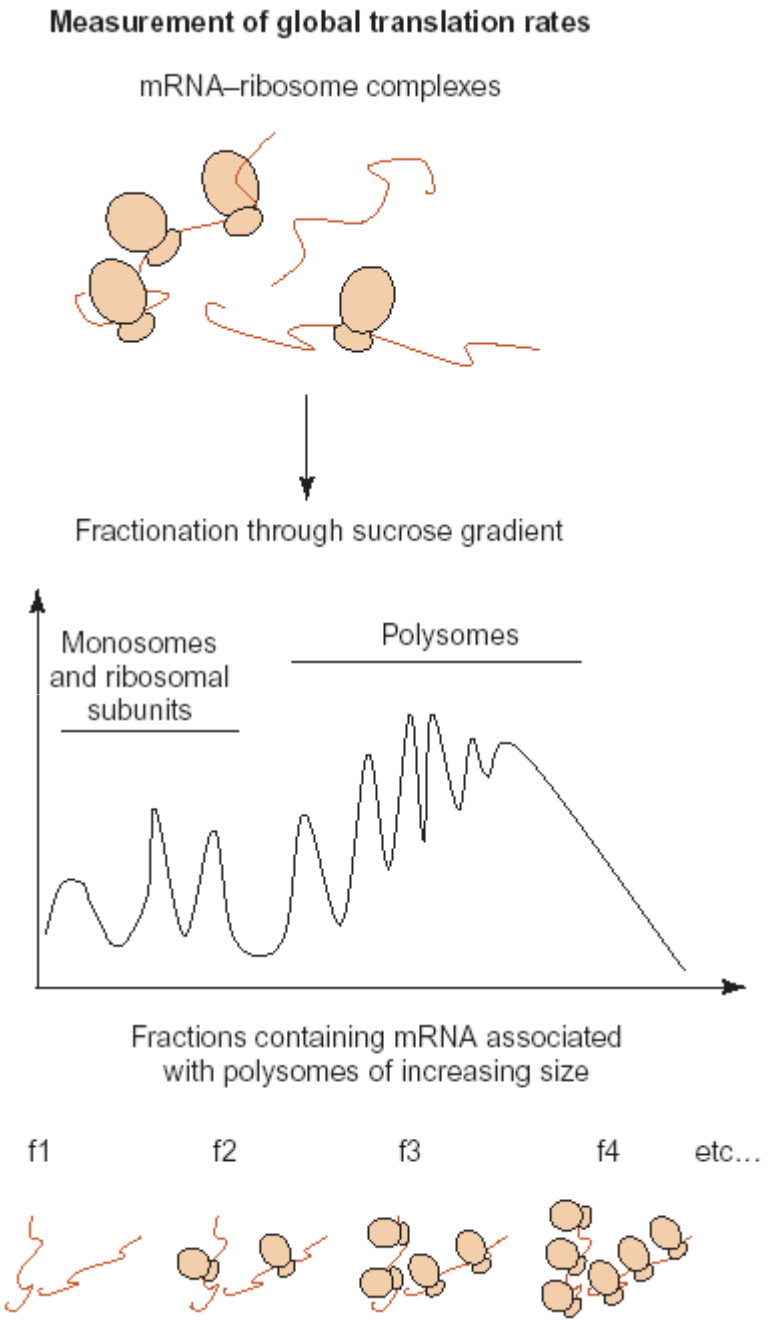
RNA extracted at time intervals

Add radioactive protection probe

RNase (ss)



RNase Protection Assay (RPA)



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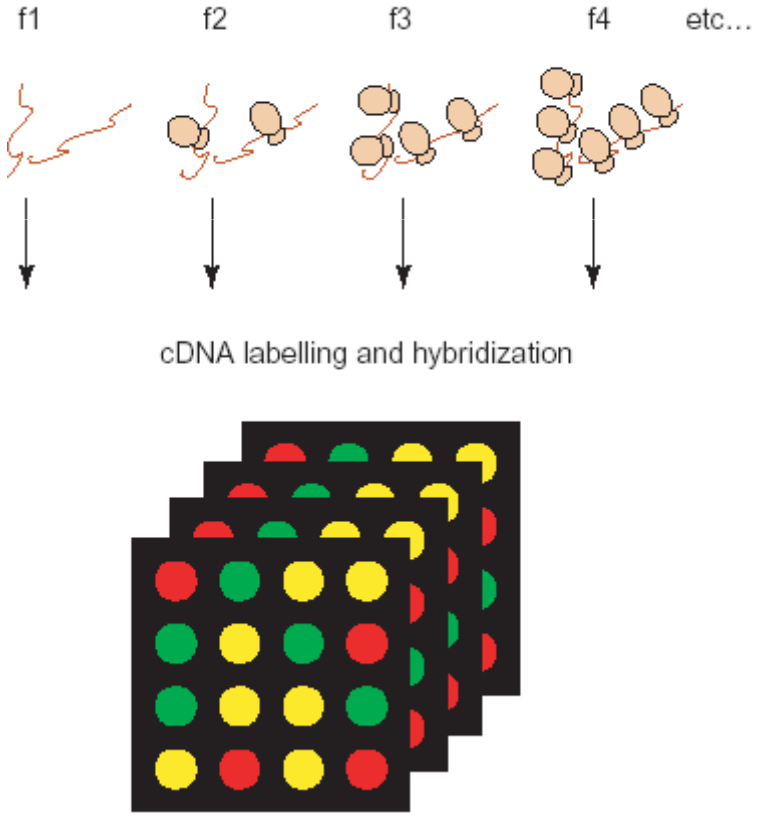
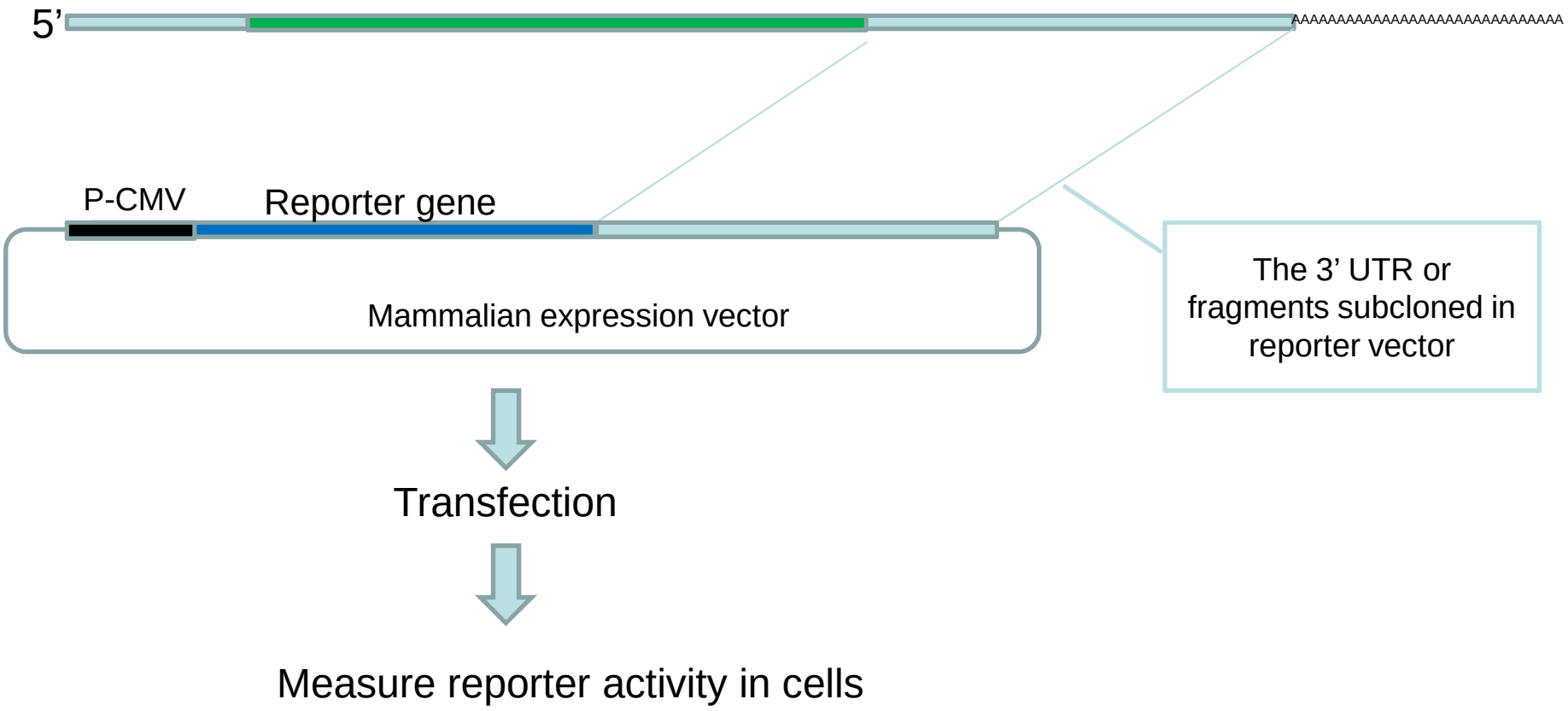


- Sequence elements in RNA are cis-acting and transferable
- Luciferase reporter assay for RNA control elements



mRNA sequence is a mosaic of sequences that are recognized by several proteins regulating their stability, translation and degradation.

There are two main classes of *trans*-regulators:

1) RNA-binding proteins

2) micro-RNA and Ago proteins (miRNA, esiRNA, piRNA).

Here we obsevre a very important step of gene regulation, since most of these activities are regulated in a cell- and development-specific fashion

One very important aspect to understand the regulatory circuitry is:

Motif finder

i.e. to identify *cis*-regulatory elements in RNAs

Letter

Decay Rates of Human mRNAs: Correlation With Functional Characteristics and Sequence Attributes

Edward Yang,[1,6] Erik van Nimwegen,[4,6] Mihaela Zavolan,[2] Nikolaus Rajewsky,[5] Mark Schroeder,[2] Marcelo Magnasco,[3] and James E. Darnell Jr.[1,7]

[1]Laboratory of Molecular Cell Biology, [2]Laboratory of Computational Genomics, [3]Laboratory of Mathematical Physics, and [4]Center for the Study of Physics and Biology, The Rockefeller University, New York, New York 10021-6399, USA; [5]Department of Biology and Courant Institute of Mathematical Sciences, New York University, New York, New York 10012, USA

Although mRNA decay rates are a key determinant of the steady-state concentration for any given mRNA species, relatively little is known, on a population level, about what factors influence turnover rates and how these rates are integrated into cellular decisions. We decided to measure mRNA decay rates in two human cell lines with high-density oligonucleotide arrays that enable the measurement of decay rates simultaneously for thousands of mRNA species. Using existing annotation and the Gene Ontology hierarchy of biological processes, we assign mRNAs to functional classes at various levels of resolution and compare the decay rate statistics between these classes. The results show statistically significant organizational principles in the variation of decay rates among functional classes. In particular, transcription factor mRNAs have increased average decay rates compared with other transcripts and are enriched in "fast-decaying" mRNAs with half-lives <2 h. In contrast, we find that mRNAs for biosynthetic proteins have decreased average decay rates and are deficient in fast-decaying mRNAs. Our analysis of data from a previously published study of *Saccharomyces cerevisiae* mRNA decay shows the same functional organization of decay rates, implying that it is a general organizational scheme for eukaryotes. Additionally, we investigated the dependence of decay rates on sequence composition, that is, the presence or absence of short mRNA motifs in various regions of the mRNA transcript. Our analysis recovers the positive correlation of mRNA decay with known AU-rich mRNA motifs, but we also uncover further short mRNA motifs that show statistically significant correlation with decay. However, we also note that none of these motifs are strong predictors of mRNA decay rate, indicating that the regulation of mRNA decay is more complex and may involve the cooperative binding of several RNA-binding proteins at different sites.

[Supplemental material is available online at www.genome.org, and also at http://genomes.rockefeller.edu/~yange.]

HepG2 cells

Actinomycin 2-3 hours

RNA extraction, labelling and → Affymetrix microarrays

Repeated on primary fibroblasts (Bud8)

Decay rates estimated for 5,245 genes.

Group of genes either short lived or long lived explored for ontology

To study the rates of mRNA degradation (“decay”) in human cells, we measured changes in mRNA levels following application of the RNA polymerase inhibitor Actinomycin D with Affymetrix U95Av2 high-density oligonucleotide arrays.

In this way, we obtained decay rate estimates for 5245 accessions

Combining the decay rate for all probe sets present in the initial and final conditions, we find that the median half-life in both cell types was ~10 h

Comparing this median half-life with the median half-lives of transcripts in yeast and bacteria, it appears that the half-life of the mRNA pool of a cell scales roughly in proportion to the length of the cell cycle: cell cycle lengths of 20, 90, and 3000 min correspond to median half-lives of 5, 21, and 600 min, respectively, for *E. coli, S. cerevisiae, a*nd human HepG2/Bud8 cells

from Yang et al., 2003.

GO codes provide a standardized, hierarchical classification for describing gene products agreed to by the public genome sequencing projects.

GO presnt.

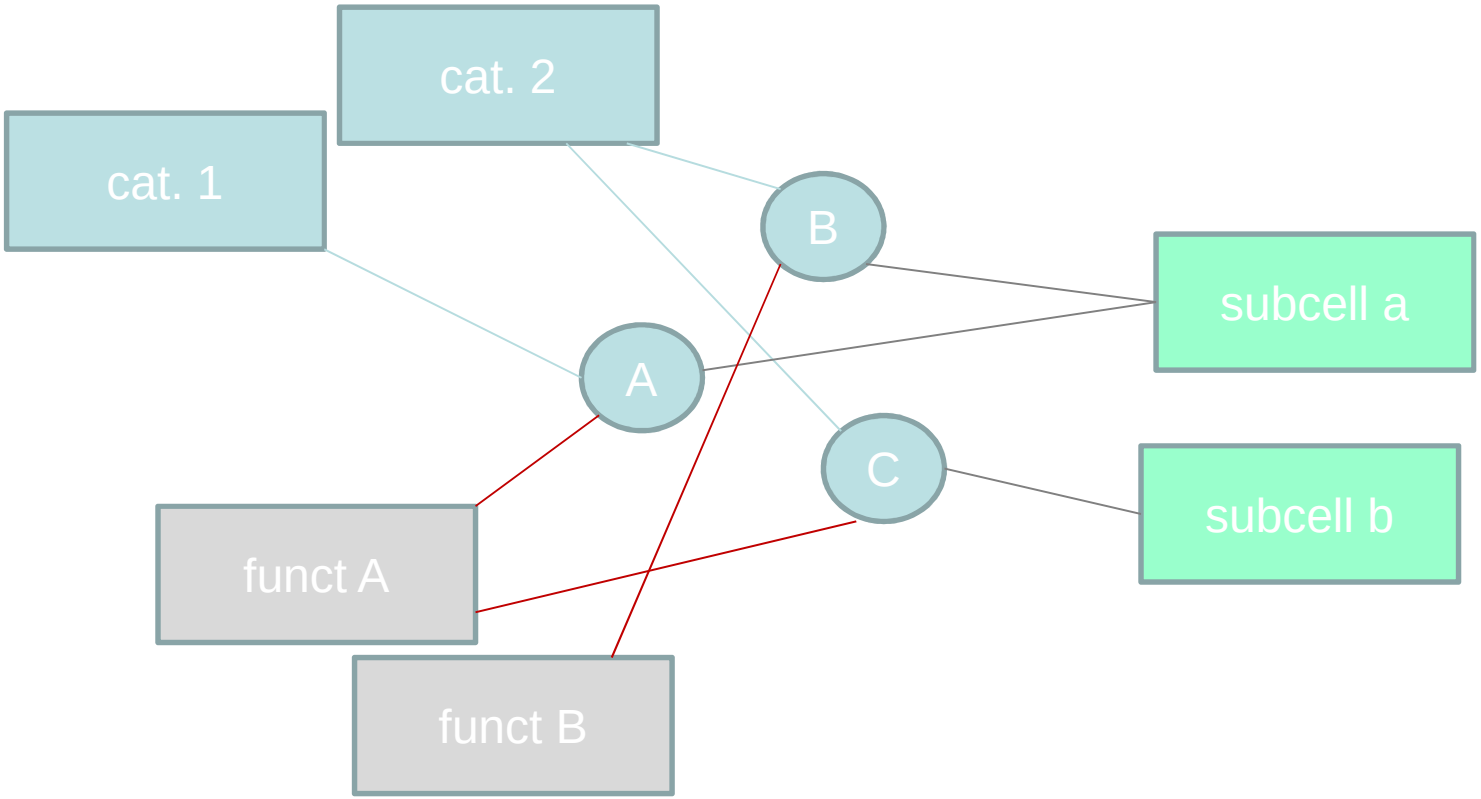
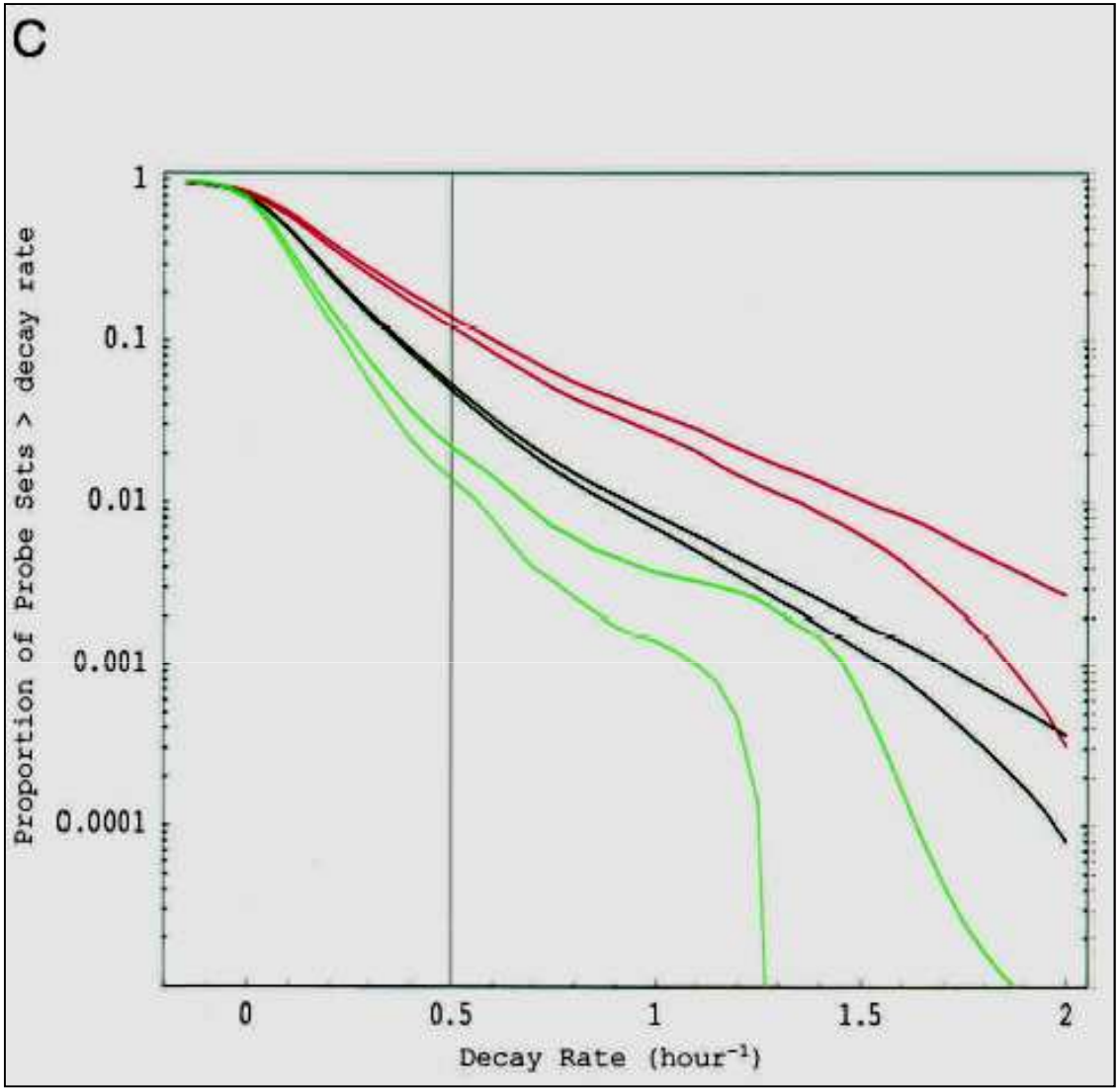


Figure 1 Functional analysis of decaying transcripts in human cells.

(C) Reverse cumulative distribution of decay rates for probe sets in different functional classes (HepG2 experiments). Decay rate r is shown horizontally, while vertically the fraction of probe sets with decay rates higher than r is plotted on a logarithmicsc ale. The pairs of lines show the 98% posterior probability intervals for the fraction at each value of r. (Red) GO process transcription; (black) all probe sets; (green) biosynthesis. The gray line indicates the decay rate r = 0.5 h1, which is our cutoff for fast decay in PFDI.



Decay rates were studied in functional groups, i.e. in G.O. categories.

from Yang et al., 2003.

As indicated in the cumulative distribution plot, a small percentage (~5%) of expressed transcripts have “fast” decay rates (which we define as $r > 0.5\ h^{-1}$ or a half-life < 2 h). A similar percentage of rapidly decaying genes was observed when we re-ran an HepG2 experiment with U95B arrays, which are predominantly expressed sequence tags.

....

Although total length of cDNA did not correlate with decay rate, we did find evidence that mRNAs with 3-UTR sequence >1 kb decayed at a significantly faster rate than shorter 3-UTRs

from Yang et al., 2003.

Upf1 Senses 3′UTR Length to Potentiate mRNA Decay

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DOI 10.1016/j.cell.2010.10.005

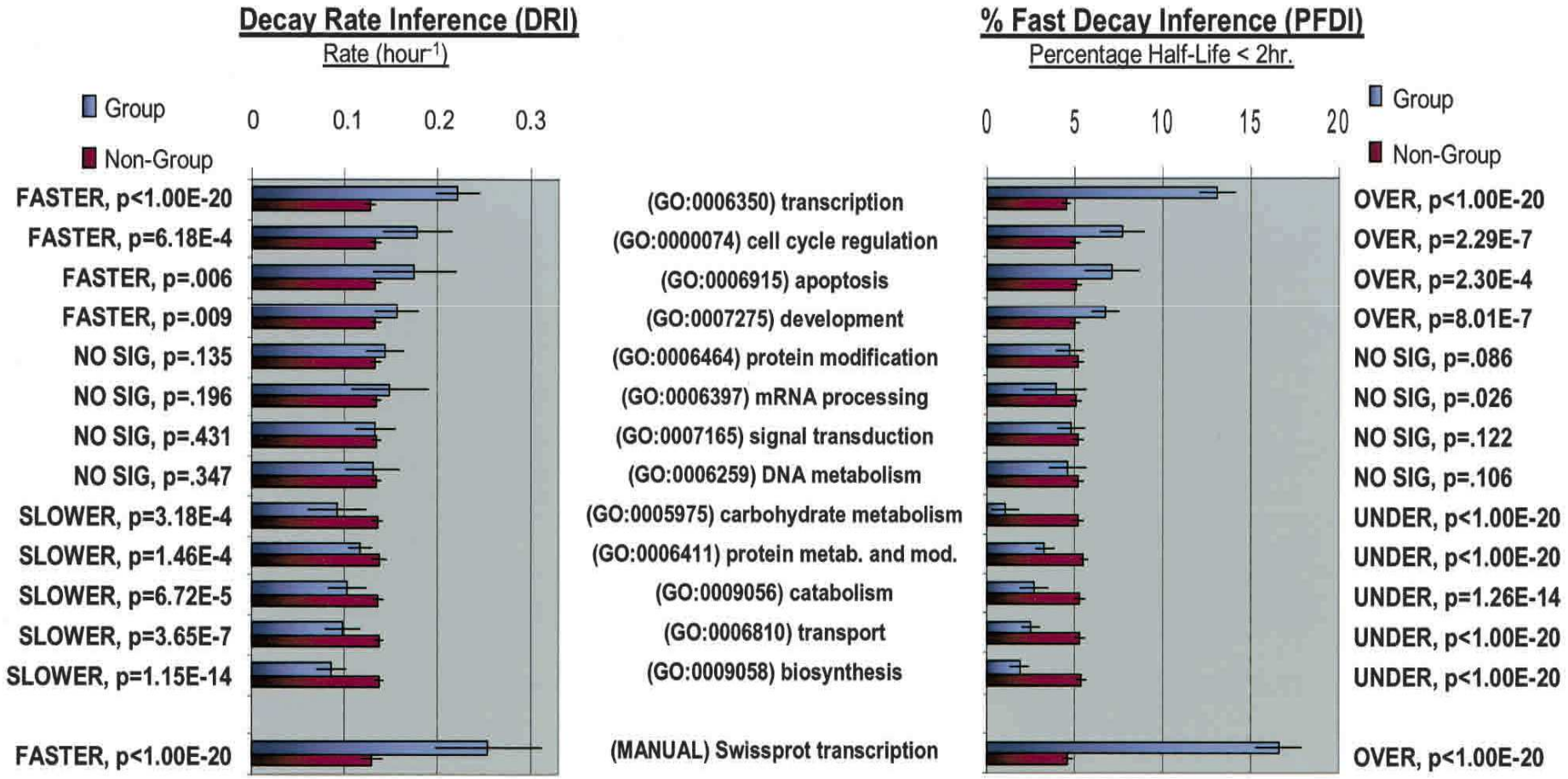
SUMMARY

The selective degradation of mRNAs by the nonsense-mediated decay pathway is a quality control process with important consequences for human disease. From initial studies using RNA hairpin-tagged mRNAs for purification of messenger ribonucleoproteins assembled on transcripts with HIV-1 3′ untranslated region (3′UTR) sequences, we uncover a two-step mechanism for Upf1-dependent degradation of mRNAs with long 3′UTRs. We demonstrate that Upf1 associates with mRNAs in a 3′UTR length-dependent manner and is highly enriched on transcripts containing 3′UTRs known to elicit NMD. Surprisingly, Upf1 recruitment and subsequent RNA decay can be antagonized by retroviral RNA elements that promote translational readthrough. By modulating the efficiency of translation termination, recognition of long 3′UTRs by Upf1 is uncoupled from the initiation of decay. We propose a model for 3′UTR length surveillance in which equilibrium binding of Upf1 to mRNAs precedes a kinetically distinct commitment to RNA decay.

Globally, the mechanisms of mRNA metabolism are still largely unknown.

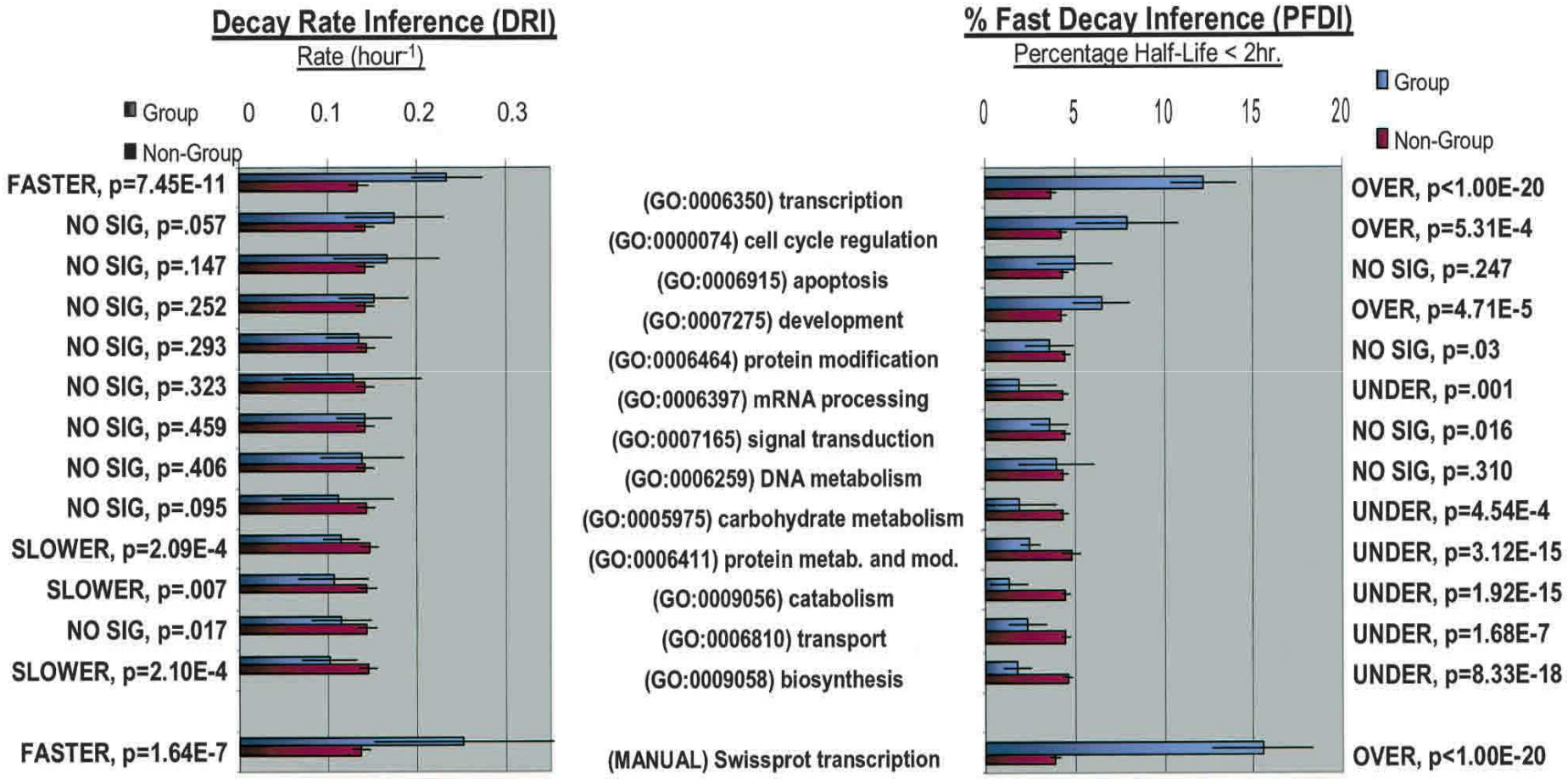
Indeed, this is today a field of very active research.

A. HepG2



from Yang et al., 2003.

B. Bud8



from Yang et al., 2003.

Known and novel motifs were examined: for each of these, DRI and PDFI is calculated and deviation from global estimated.

HepG2 motif summary

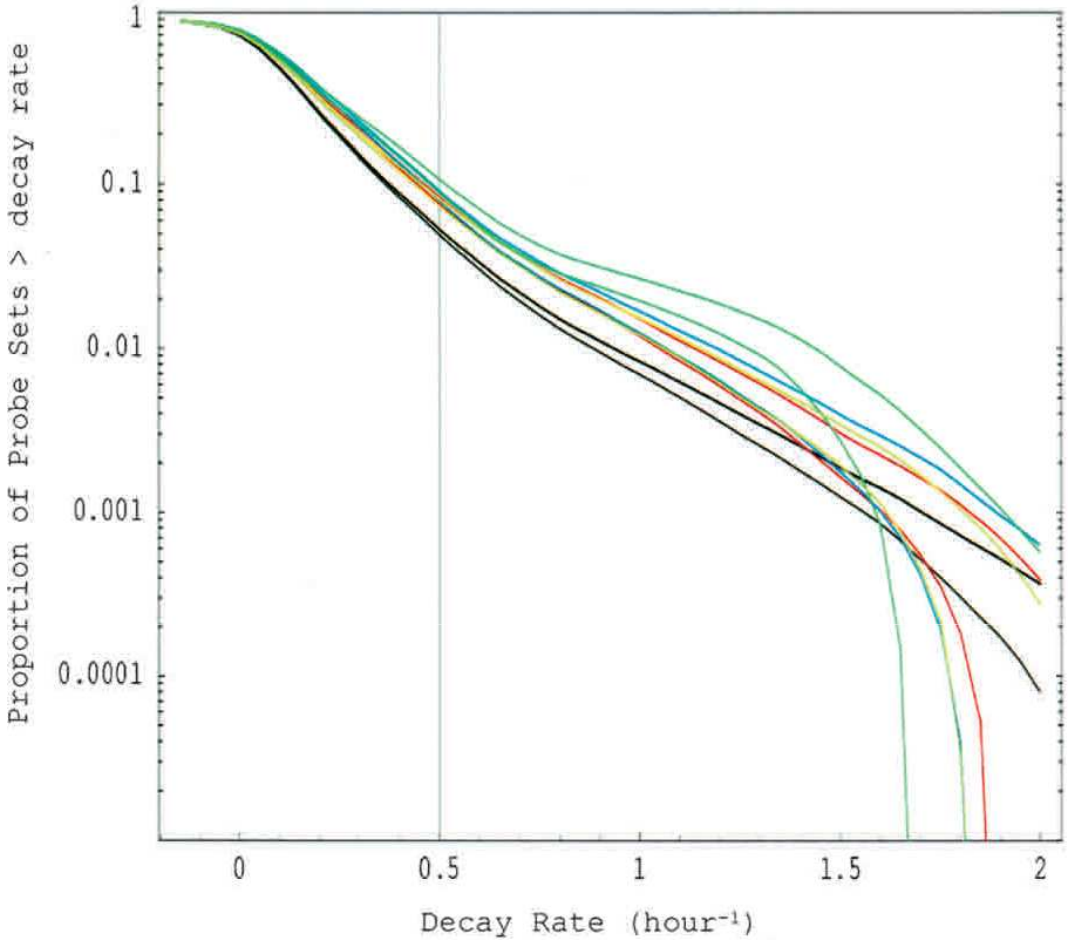
		DRI				PFDI			
		p-value for In-Group rate change				p-value for In-Group Representation change			
Motif	**Sequence**	5'UTR	ORF	3'UTR	WHOLESEQ	5'UTR	ORF	3'UTR	WHOLESEQ
Described Motifs									
1	[AT]ATTTA[AT]		2.02E-12	0	0	2.95E-06	0	0	0
2A	ATTTATTTATTTATTTATTTA		na				na		
2B	ATTTATTTATTTATTTA		na				na		
2C	[AT]ATTTATTTATTTA[AT]		na				na		
2D	[AT]{2}ATTTATTTA[AT]{2}		na				na	0.009	
2E	[AT]{4}ATTTA[AT]{4}		3.50E-04	1.13E-13	1.38E-14		1.70E-14	0	0
MEG	TTATTTATT			2.57E-07	1.00E-07			7.50E-20	1.00E-20
MEGSHORT	TATTTAT		5.03E-04	0	0	8.75E-04	0.001	0	0
Undescnbed Motifs									
H1	TTTTTTT			0	3.50E-20	2.33E-04	0.001	0	0
H2	TTTTTAAA	3.83E-04	4.84E-04	0	0	1.93E-10	2.03E-10	0	0
H3	TTGTAAATA			4.77E-11	6.96E-10		4.43E-05	0	0
B1	TTTTAAAT		1.48E-06	1.52E-11	1.57E-13		0	0	0
B2	TTTTAATTT				0.005	*0.007*	0.008	7.24E-04	1.94E-04
B3	AAATATTTT		0.004	6.05E-09	5.11E-10		6.33E-05	0	0
B4	AATATTTTT			3.43E-09	6.77E-10		7.94E-05	2.00E-20	0
H-1	CCGCCTC		0.005					*2.86E-05*	
H-2	CCAGCCTC		1.93E-08		4.18E-04	*2.89E-04*	0	*8.56E-11*	9.39E-07
B-1	GGGCCTGG					*0.004*	*0.007*		*5.65E-04*
B-2	CCCAGCCCC		7.72E-04				2.96E-06	*1.78E-07*	

from Yang et al., 2003.

Bud8 motif summary

		DRI				PFDI			
		p-value for In-Group rate change				p-value for In-Group Representation change			
Motif	Sequence	5'UTR	ORF	3'UTR	WHOLESEQ	5'UTR	ORF	3'UTR	WHOLESEQ
Described Motifs									
1	[AT]ATTTA[AT]		0.010	1.92E-13	6.14E-12		0.002	0	0
2A	ATTTATTTATTTATTTATTTA		na	na			na	na	
2B	ATTTATTTATTTATTTA		na	na			na	na	
2C	[AT]ATTTATTTATTTA[AT]		na				na	*0.005*	*9.96E-04*
2D	[AT]{2}ATTTATTTA[AT]{2}		na				na		
2E	[AT]{4}ATTTA[AT]{4}			5.50E-06	2.18E-05			4.22E-16	1.44E-13
MEG	TTATTTATT			0.001	0.004		*3.38E-06*	4.44E-11	1.17E-08
MEGSHORT	TATTTAT			8.05E-10	1.40E-07			0	2.22E-15
Undescribed Motifs									
H1	TTTTTTT			3.32E-07	1.22E-06		*0.001*	0	0
H2	TTTTTAAA			1.39E-07	9.70E-09	0.009		0	0
H3	TTGTAAATA			3.45E-06	7.95E-06		6.20E-05	3.44E-16	2.70E-15
B1	TTTTAAAT		0.004	0.001	1.50E-04		3.78E-08	7.61E-09	2.83E-10
B2	TTTTAATTT			0.005	0.003		0.009	1.06E-05	1.37E-06
B3	AAATATTTT							7.31E-06	2.78E-06
B4	AATATTTTT			0.003	0.007			7.70E-07	3.96E-06
H-1	CCGCCTC						0.009		0.008
H-2	CCAGCCTC						0.001	*0.001*	
B-1	GGGCCTGG					*1.84E-04*	0.007		
B-2	CCCAGCCCC			*0.003*				*8.51E-05*	

from Yang et al., 2003.



from Yang et al., 2003.

(*C) Reverse cumulative distribution of decay rates for* probe sets from genes that contain particular sequence motifs in their 3-UTR (HepG2 experiment). Decay rate *r is shown horizontally, while vertically the fraction of probe sets with decay rates higher than r is* plotted on a logarithmicsc ale. The pairs of lines show the 98% posterior probability intervals for the fraction at each value of *r. (Red) Motif 1; (blue) motif MEGSHORT; (green) Motif 2E; (light green) Motif H1;* (black) all probe sets. “Described” AU-rich decay motifs (1–2E, MEG, MEGSHORT) and “undescribed” motifs were derived from the sources mentioned in Methods.

....
Together, these observations show that the examined RNA motifs correlate with shifts in the distribution of decay rates, but that they do not reliably predict turnover behavior. It thus seems that the regulation of mRNA decay is more complicated and might involve combinatorial interactions, that is, cooperative binding between different RNAbinding proteins that bind at different sites in the mRNA. This might also explain why the effect on decay rate is context dependent for certain motifs (such as H-2).

from Yang et al., 2003.

Recent systematic analysis of short-living and long-living mRNA demonstrates also RNA elements that function on a cell-specific basis.

Systematic Analysis of *Cis*-Elements in Unstable mRNAs Demonstrates that CUGBP1 Is a Key Regulator of mRNA Decay in Muscle Cells

Jerome E. Lee[1☯], Ju Youn Lee[3☯], Jeffrey Wilusz[1,2], Bin Tian[3¶], Carol J. Wilusz[1,2*¶]

1 Program in Cell and Molecular Biology, Colorado State University, Fort Collins, Colorado, United States of America, **2** Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, Colorado, United States of America, **3** Department of Biochemistry and Molecular Biology, New Jersey Medical School, University of Medicine and Dentistry of New Jersey, Newark, New Jersey, United States of America

Abstract

Background: Dramatic changes in gene expression occur in response to extracellular stimuli and during differentiation. Although transcriptional effects are important, alterations in mRNA decay also play a major role in achieving rapid and massive changes in mRNA abundance. Moreover, just as transcription factor activity varies between different cell types, the factors influencing mRNA decay are also cell-type specific.

Principal Findings: We have established the rates of decay for over 7000 transcripts expressed in mouse C2C12 myoblasts. We found that GU-rich (GRE) and AU-rich (ARE) elements are over-represented in the 3'UTRs of short-lived mRNAs and that these mRNAs tend to encode factors involved in cell cycle and transcription regulation. Stabilizing elements were also identified. By comparing mRNA decay rates in C2C12 cells with those previously measured for pluripotent and differentiating embryonic stem (ES) cells, we identified several groups of transcripts that exhibit cell-type specific decay rates. Further, whereas in C2C12 cells the impact of GREs on mRNA decay appears to be greater than that of AREs, AREs are more significant in ES cells, supporting the idea that *cis* elements make a cell-specific contribution to mRNA stability. GREs are recognized by CUGBP1, an RNA-binding protein and instability factor whose function is affected in several neuromuscular diseases. We therefore utilized RNA immunoprecipitation followed by microarray (RIP-Chip) to identify CUGBP1-associated transcripts. These mRNAs also showed dramatic enrichment of GREs in their 3'UTRs and encode proteins linked with cell cycle, and intracellular transport. Interestingly several CUGBP1 substrate mRNAs, including those encoding the myogenic transcription factors *Myod1* and *Myog*, are also bound by the stabilizing factor HuR in C2C12 cells. Finally, we show that several CUGBP1-associated mRNAs containing 3'UTR GREs, including *Myod1*, are stabilized in cells depleted of CUGBP1, consistent with the role of CUGBP1 as a destabilizing factor.

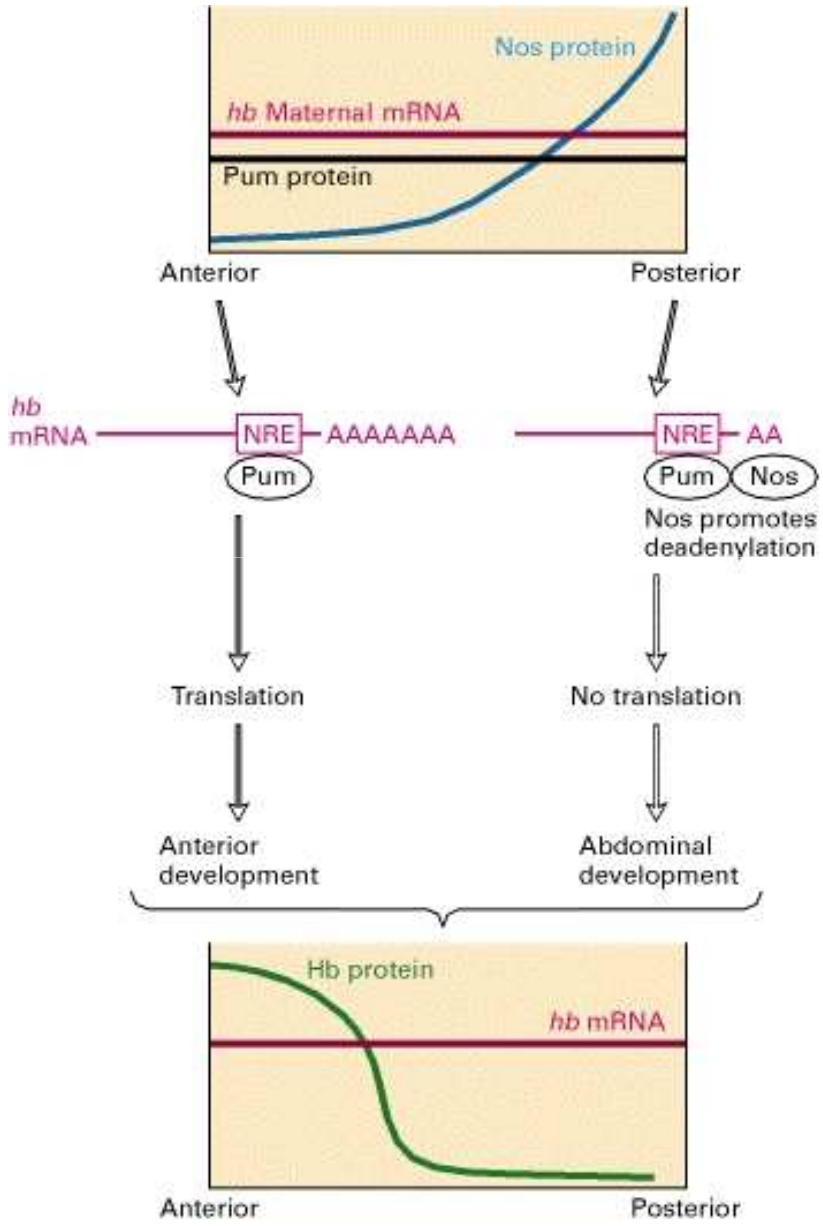
Conclusions: Taken together, our results systematically establish *cis*-acting determinants of mRNA decay rates in C2C12 myoblast cells and demonstrate that CUGBP1 associates with GREs to regulate decay of a wide range of mRNAs including several that are critical for muscle development.

Motifs imparting shorter or longer half-life may represent protein-binding elements or miRNA targets. These latter are quite easy to recognize by mapping the sequences back to the genome.

The “other way around” story is, given a RBP (a protein presenting RRM motifs), to identify all mRNA that are bound to it (and possibly affected).

The *Pumilio* protein was originally identified (and named) in Drosophila embryo, where in complex with *nanos* protein it inhibits translation of the *hunchback* mRNA at the posterior pole, giving rise to the gradient of *hunchback* protein that is one of the primary regulators of segmentation.

Homologs of Pumioio are found in all organism and are called Puf.
Humans have 2 genes



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Comparative Analysis of mRNA Targets for Human PUF-Family Proteins Suggests Extensive Interaction with the miRNA Regulatory System

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Assigned paper

Abstract

Genome-wide identification of mRNAs regulated by RNA-binding proteins is crucial to uncover post-transcriptional gene regulatory systems. The conserved PUF family RNA-binding proteins repress gene expression post-transcriptionally by binding to sequence elements in 3′-UTRs of mRNAs. Despite their well-studied implications for development and neurogenesis in metazoa, the mammalian PUF family members are only poorly characterized and mRNA targets are largely unknown. We have systematically identified the mRNAs associated with the two human PUF proteins, PUM1 and PUM2, by the recovery of endogenously formed ribonucleoprotein complexes and the analysis of associated RNAs with DNA microarrays. A largely overlapping set comprised of hundreds of mRNAs were reproducibly associated with the paralogous PUM proteins, many of them encoding functionally related proteins. A characteristic PUF-binding motif was highly enriched among PUM bound messages and validated with RNA pull-down experiments. Moreover, PUF motifs as well as surrounding sequences exhibit higher conservation in PUM bound messages as opposed to transcripts that were not found to be associated, suggesting that PUM function may be modulated by other factors that bind conserved elements. Strikingly, we found that PUF motifs are enriched around predicted miRNA binding sites and that high-confidence miRNA binding sites are significantly enriched in the 3′-UTRs of experimentally determined PUM1 and PUM2 targets, strongly suggesting an interaction of human PUM proteins with the miRNA regulatory system. Our work suggests extensive connections between the RBP and miRNA post-transcriptional regulatory systems and provides a framework for deciphering the molecular mechanism by which PUF proteins regulate their target mRNAs.